Physiological response of Garlic (*Allium sativum*) to elevated tropospheric ozone in high altitude region of Western Ghats, Tamil Nadu, India

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Abstract

A pot culture study was conducted at Horticultural Research Station, Ooty, to assess the effect of ground-level ozone on physiology in garlic plants and find out suitable remedial measures against ground-level ozone. The potting soil was acidic with very low salt concentration, very high in organic carbon, medium in nitrogen, phosphorus and high in potassium. Elevated ozone levels (150ppb and 200ppb) had significantly reduced the garlic plant chlorophyll content, stomatal conductance, photosynthetic rate, pungency and total soluble solids. The highest chlorophyll content ($33.97\mu\text{molm}^{-2}$) was observed under ambient ozone level (T_1), and lowest chlorophyll content ($12.68\mu\text{molm}^{-2}$) was observed in elevated ozone exposure at 200 ppb (T_3), highest stomatal conductance ($0.45\text{mmol m}^{-2}\text{s}^{-1}$) was recorded in Ambient Ozone level+foliar spray 3%Panchagavya (T_4), and lowest stomatal conductance ($0.11\text{mmolm}^{-2}\text{s}^{-1}$) was observed in elevated ozone exposure at 200 ppb (T_3). Since the elevated ozone had a significant reduction in photosynthetic rate in garlic, lowest was observed ($0.82\mu\text{molCO}_2 \text{ s}^{-1}\text{m}^{-2}$) in T_3 -Elevated ozone exposure at 200 ppb and the highest photosynthetic rate ($3.02\mu\text{molCO}_2 \text{ s}^{-1}\text{m}^{-2}$) was observed in treatment T_4 - Ambient Ozone level+foliar spray 3%Panchagavya after a week. When coming to quality of garlic bulbs, highest pungency content was found in Ambient Ozone level+foliar spray 0.1%Ascorbic acid and lowest was observed in Elevated ozone exposure at 200 ppb (T_3) furthermore, in observing garlic quality where total soluble solids (TSS) showed that the treatment Ambient Ozone level+foliar spray 3%Panchagavya (40.00°Brix) as highest and treatment Elevated ozone exposure at 200 ppb (T_3) recorded the lowest. Thus, tropospheric ozone has detrimental impact on physiological responses, which will reduce crop growth and yield. The ozone protectants helped in scavenging the O_3 from apoplast of the crops. Among the ozone protectants, neem oil acted as a good ozone scavenger followed by ascorbic acid and panchagavya to improve the physiological response of garlic plants under elevated tropospheric ozone levels.

1. Introduction

Air pollution is a severe concern to humans, crops, and animals and primary significant emitter is human beings. The primary air pollutants such as sulfur dioxide (SO_2), carbon monoxide (CO), methane (CH_4), non-methane volatile organic compounds (NMVOC) and nitrogen oxides (NO_x) formed the tropospheric ozone, a secondary air pollutant by the oxidation of CO , CH_4 or NMVOCs in presence of NO_x (nitrogen oxides) (Wedow et al., 2021) and the concentration of ozone increased due to industrialization (Gulke and Heath, 2020). Surface level ozone concentration is increasing in Asia and decreasing or stagnating in Europe and North America, which possess great threats to food production and food quality in Asia (Feng et al., 2021). The endemic richness area such as Atlantic islands, Mediterranean Basin, equatorial Africa, Ethiopia, the Indian coastline, the Himalayan region, southern Asia, and Japan are under high risk of biodiversity loss due to increasing concentration of surface level ozone by 2100 under RCP 2.6, RCP 4.5 and RCP 8.5 climate change scenarios (Agathokleous et al., 2020). There was a significant correlation of

AOT 40 ozone exposure and human health, food production in China and in the year 2015, China lost 7% of the GDP due to ozone exposure (Feng et al., 2019).

In India, tropospheric ozone level is higher during winter, in this case, transport and residential sectors are most leading source for increasing tropospheric levels whereas, the northern parts of India contribute more compare to southern parts of India (Gao *et al.*, 2019). Ozone is a short-lived pollutant, which significantly declines the growth, yield and productivity of crops globally (Ainsworth, 2016), when its concentration increased in the atmosphere's troposphere. Ozone enters into the plants through stomata and forms reactive oxygen species (ROS) directly by reacting with organic molecules present in apoplast, as well as induction of endogenous formation of ROS that can collectively cause cell damage and programmed cell death (Ainsworth, 2016; Li et al., 2017) when O₃ concentration exceeds the critical levels (Kanagendran *et al.*, 2017). Even, present surface level ozone concentration is causing damage to sensitive crops in Asia, North America and Europe and the expected yield loss due to projected ozone concentration under optimistic emission scenario would be 0.1 to 11% wheat, soya bean and maize by 2030 (Emberson, 2020).

Increase in concentration of ozone causes leaf injury, senescence, abscission, stomatal closure leading to reduction of photosynthesis, reduction of root growth, reduction of leaf growth, reduction of plant biomass and reduction of phloem translocation efficiency, which resulted in a significant decrease in crop yield (Wilkinson et al., 2012). According to the effect of ozone, few crops has been classified so far as sensitive (Wheat, watermelon, pulses, cotton, turnip, tomato, onion, soybean and lettuce), moderately sensitive (Potato, sugar beet, potato, oilseed rape, tobacco, rice, grape, broccoli and maize) and tolerant (Barley, plum and strawberry) (Emberson et al., 2009). The crops exposed to higher concentrations of ozone show some common symptoms on foliar i.e., ozone injury. Acute injuries are flecking and stippling, whereas the chronic injuries are bronzing, chlorosis and premature senescence. The estimated wheat loss due to ozone is 39.7%, 31.8% and 26.8% in Developed Countries, Upper Middle-Income Countries and Territories and Lower Middle-Income Countries and Territories respectively (Mills et al., 2018). Furthermore, the increase in ozone concentration would alter the foliar chemical composition and thereby changing plant - insect interaction and finally affects the ecosystem services (Agathokleous et al., 2020).

In increasing radiative forcing, O₃ as a greenhouse gas modifies water and carbon exchange between flora and atmosphere regionally and globally by affecting transpiration and photosynthesis of plants (Lombardozzi et al., 2012). The O₃ has potential to destruct photosynthesis in several ways when it enters the leaf via stomata, it oxidizes the cellular membrane by altering mesophyll cells, thus lowering Rubisco activity and carbon fixation. This results in reducing chlorophyll content (Kulshrestha and Saxena, 2016). The regulation of photosynthesis and transpiration is modified by stomatal conductance. The direct way of affecting the plants is by changing guard cell turgor pressure and signaling pathways and indirectly by stomatal closure in case of a rise in intercellular CO₂ concentration (Lombardozzi et al., 2013). In few studies with respect to chronic O₃ exposure, there was increase in stomatal conductance (Lombardozzi et al., 2013). Photosynthesis is directly proportional to chlorophyll content and net

assimilation rate (Kobayakawa and Imai, 2017), hence, declining of photosynthetic rates resulted in lowering of biomass in crops (Chen et al., 2018) due to increased level of tropospheric ozone concentration. The negative effects of elevated tropospheric O₃ concentration include decreased plant growth and altering the plant metabolism that significantly reduced crop yield (Saxena et al., 2019).

Ozone sensitivity varies across species due to species-specific biochemical, physiological and morphological traits (Li et al., 2016), stomatal conductance, and leaf antioxidant capacity (Brosche *et al.*, 2010; Fares et al., 2013). An increase in the concentration of ozone suppressed photosynthetic activity and stomatal conductance, resulting in premature leaf fall and reduced biomass content that showed changes in physiology and growth of crops (Tetteh et al., 2015). The net photosynthesis reduction in wood plants also occurred due to elevated levels (Li et al., 2016). Reductions in stomatal conductance (gs), net photosynthetic CO₂ assimilation and carboxylation efficiency had all been associated with O₃ exposure to sorghum (Li et al., 2021). A decline in photosynthetic rate in O₃ exposed plants was associated with damage to the photosynthetic machinery that leads to reduced fixation and increased CO₂ concentration (Ci), resulting in reduced stomatal conductance (gs) (Yadav et al., 2020).

Ozone protectant studies are also being carried out globally to reduce the ozone impact on crops and improve crop yield. A unique preparation of Panchagavya is by both water (milk, urine, curd, dung, tender coconut water) and fat (ghee, milk with fat) products (Boomiraj and Christopher 2004) where polar and non-polar natural antioxidants are possibly produced by panchagavya that are essential for intracellular and intercellular parts of leaves in scavenging oxidative stress of elevated ozone. Furthermore, it has antioxidant potential by the experiment conducted by Athavale et al., 2012 with total phenols, FRAP and DPPH assays. Panchagavya were studied as ozone protectants in cauliflower (Sethupathi et al., 2018) and different plants and tree species of shola forest (Murugaragavan *et al.*, 2018). Similarly, neem oil, which used in the study have highest percentage of antioxidant activity imputed to total phenol content under ethanol extract and it generates more free radicals, which have ability to inhibit highest percentage of DPPH radical. (Nahak and Sahu, 2011). Neem oil as ozone protectants studies in different crops like cauliflower (Sethupathi et al., 2018) and in different plants and tree species of shola forest (Murugaragavan *et al.*, 2018). Ascorbic acid as a source of vitamin c plays a vital role to reduce the ozone impact in plants such as groundnut (Chaudhary *et al.*, 2020), Soyabean (Jiang *et al.*, 2018), Wheat (Fatima et al., 2019), Arabidopsis (Bellini and Tullio, 2019), Cauliflower (Sethupathi et al., 2018). The application of ascorbic acid, neem oil, and panchagavya was done to assess their capability to reduce the ozone impact on garlic crops in this study. Thus, the current study intended to study physiological responses and quality of garlic to elevated different ozone levels.

2. Materials And Methods

The permission for experimental site as well as the use of Garlic Variety Ooty-1 (seed material) was granted and provided by Tamil Nadu Agricultural University advisory committee for the student research.

2.1 Status of trace gas level at Ooty

The study area, Ooty located in the District of Nilgiris, a fertile mountain range in Indian state of Tamil Nadu., represents a fresh, clean and pleasant environment with highly subdued human activity. Dense forests, lofty mountains, extensive tea and coffee plantation and sprawling grasslands characterize the location. The Nilgiris Hills form a part of a larger chain of mountains known as the Western Ghats along the western side of India, which is one among the eight hottest hotspots of biological diversity in the world. Due to anthropogenic activities the climate is changing drastically at Nilgiris District. Seasonal variation has been observed based on the meteorological data recorded. The maximum temperature in summer has increased to 26⁰C (Normal 24⁰C) and winter temperature 4⁰C (Normal 2⁰C). Black carbon level in summer has increased to 6000 ng (normal level 50-100 ng) in this District. While comparing to other regions, the carbon level is very high in this District. The measured Ozone and NO_x concentrations for a period of 8 years between 2010 and March 2018 showed seasonal mean values (ppb) of 53.45, 19.81, 27.2 and 42.81 for summer, Monsoon, post-monsoon and winter, respectively. A monthly maximum NO_x value was observed during the summer month of April (1.85 ppb), whereas a minimum during the monsoon month of August (0.19 ppb). Monthly maximum SO₂ observed during summer months such as March and April recorded 1.0, and 0.6 ppb, respectively and the minimum value of about 0.10 ppb was observed during November. The average CO value was found to be 160.92 ppb. Strong diurnal and seasonal mean values of trace gases during summer are attributed mainly to the ABL dynamics, advection pathways and source types. The HYSPLIT back-trajectory analysis indicates the dominant easterly advection contributing to the seasonal high value of trace gases during the summer. Due to climate change, the monsoon and pre-monsoon patterns are changed. This seasonal variation directly affects the crop yield, quality, cropping pattern and the incidence of pests and diseases.

2.2. Experimental Site and O₃ measurement

The experiment was conducted in Western Ghats subtropical highland climatic region at 11.4°N, 76.7°E at an altitude of 2520m mean sea level from September to December 2018 in the experimental Woodhouse Farm Horticultural Research Station, Tamil Nadu Agricultural University, Ooty, Nilgiris District.

Based on UV absorption, the ambient ozone measurements were continuously monitored using an ozone analyzer (Thermo Fischer U.S.A Model 49i in the Fig.1) from February 2010. It measures the amount of ozone in the air from 0.05 ppb concentrations up to 200 ppm. The analyzer aspirates air from a height of about 5 m above the ground level through a Teflon tube with a flow rate of about 1 L per minute. The minimum detection limit of the analyzer is about 1 ppb. Calibration is done at frequent intervals using zero air and known span values. The ambient ozone concentration in the experimental site of Ooty recorded from 2010, in which minimum of 10ppb and a maximum of 120ppb were recorded. The highest ambient O₃ are found in the years 2015 and 2016, in which it peaks from 100.446 to 123 ppb and 100.154 to 109.199 ppb were recorded. The unforeseen increase in the O₃ concentration may be expected to escalate about 150ppb and 200ppb in the years ahead. Hence this intended the authors to study with elevated 150ppb and 200ppb of O₃ levels.

2.3. Exposure design

The electric supply was provided to connect the corona discharge through ground cable for carrying out the experiment from the Ozone generator. For the uniform distribution of ozone, which is fumigated, the axial flow fans were fixed to the open top chamber. As depicted in fig. 2, Garlic had been grown in prepared pots and watered regularly. The plants were maintained uniformly up to the most critical stage of garlic outside the chamber. Only during the critical stages, the crops were exposed to 150 and 200 ppb (Elevated level of Ozone) of ozone and fumigated at the rate of 4 hours'day⁻¹ within the chamber.

2.4. Time of exposure

Growth of a garlic plant occurs in several stages, *viz.*, clove sprouting and establishment, bulb initiation, bulb development and bulb maturation. The bulb initiation stage of garlic is considered the essential critical stage since most of the significant physiological and growth effects occur during the later stages of crop development. So, the bulb initiation stage of garlic has been taken for elevated levels of ozone (O₃) exposures. Exposure to ozone between 40-100 ppb may cause different injury patterns in different crops according to its sensitivity. Based on this concept, the following treatments were fixed.

2.5. Treatments details

The potted experiment of garlic treated with two different levels of ozone (150ppb and 200ppb) under open top chambers (OTCs) with twelve treatments arranged in factorial completely randomized block design (FCRD) with three replications are as follows

T₁-Ambient ozone level,

T₂- Elevated ozone exposure at 150 ppb,

T₃- Elevated ozone exposure at 200 ppb,

T₄-Ambient ozone level + foliar spray 3% Panchagavya,

T₅-Ambient ozone level + foliar spray 3% Neem oil,

T₆-Ambient ozone level + foliar spray 0.1% Ascorbic acid,

T₇-Elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya,

T₈-Elevated ozone exposure at 150 ppb + foliar spray 3% Neem oil,

T₉- Elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid,

T₁₀- Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya,

T₁₁-Elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil,

T₁₂ -Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid.

2.6. Chlorophyll content meter

The relative chlorophyll concentration is determined using the instrument CCM-200 plus (chlorophyll content meter) designed by optic sciences (USA). The chlorophyll content meter readings were recorded regularly at the time of garlic exposure to elevated levels of ozone.

2.7. Photosynthetic rate and Stomatal conductance

The fumigation study with elevated levels of ozone on plants in pots where the crop's physiological responses were considered essential criteria in crop growth and yield. Hence, the LCpro-SD portable photosynthetic system (PPS), designed by ADC BioScientific Ltd., was used to measure the physiological parameters, i.e., photosynthetic rate and stomatal conductance. Daily readings were recorded every day at the time of exposure of crops under different concentrations of ozone. The above characters were statistically analyzed using SSPS version 16 in a one-way analysis of variance (ANOVA). The significant differences between the means were determined with Duncan's multiple range test to assess tropospheric ozone's impact on garlic's physiological response.

2.8. Pungency (pyruvate analysis)

To determine the content of naturally occurring pyruvate and carbonyl compounds, DNPH (2,4-dinitrophenylhydrazine) is used. The pyruvate content was determined using a colorimetry spectrophotometer at 420 nm (Thermo Spectronic Genesys 20; ThermoFisher Inc., Waltham, USA) (Schwimmer and Guadagni, 1962).

2.9. Total Soluble Solids (⁰Brix)

Total soluble solids had been determined by using the hand-held refractometer after harvesting the bulbs. The refractometer was standardized by using distilled water. 1 to 2 drops of aliquot juice were dropped onto the prism and thus the readings were noted and washed with distilled water every time after each sample and dried with tissue paper

3. Results

3.1. Physiological parameters

3.1.1. Chlorophyll content

The chlorophyll content of garlic was not significantly differed initially up to four days after exposure. From the fifth day onwards, a significant difference was observed in the chlorophyll content of garlic to elevated ozone exposure (Fig. 3). The chlorophyll content of garlic was significantly reduced due to elevated ozone exposure at 150 and 200 ppb. Among the treatments, the highest chlorophyll content

during 7th day was observed in ambient ozone level (T_1) ($33.97 \mu\text{mol m}^{-2}$) followed by T_4 - Ambient Ozone level + foliar spray 3% Panchagavya ($32.70 \mu\text{mol m}^{-2}$), which were on par with T_6 - Ambient Ozone level + foliar spray 0.1% Ascorbic acid ($31.03 \mu\text{mol m}^{-2}$) and T_5 - Ambient Ozone level + foliar spray 3% Neem oil ($27.13 \mu\text{mol m}^{-2}$) and the lowest chlorophyll content was recorded in T_3 - Elevated ozone exposure at 200 ppb ($12.68 \mu\text{mol m}^{-2}$) where the second lowest value was observed in T_{11} - Elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil ($15.03 \mu\text{mol m}^{-2}$) which were on par with T_2 - Elevated ozone exposure at 150 ppb ($16.93 \mu\text{mol m}^{-2}$) followed by T_{12} - Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid ($17.03 \mu\text{mol m}^{-2}$), T_8 - Elevated ozone exposure at 150 ppb + foliar spray 3% Neem oil ($18.53 \mu\text{mol m}^{-2}$), T_9 - Elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid ($19.23 \mu\text{mol m}^{-2}$) and T_{10} - Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya ($19.57 \mu\text{mol m}^{-2}$) with respect to chlorophyll content in garlic.

3.1.2. Stomatal conductance:

The garlic stomatal conductance was significantly affected by elevated ozone exposure during the critical stage (bulb initiation stage) (Table. 1); even one day after exposure, the highest stomatal conductance was observed in ambient ozone level + foliar spray 0.1% Ascorbic acid (T_6) ($0.35 \text{ mmol m}^{-2} \text{ s}^{-1}$) followed by T_4 - Ambient Ozone level + foliar spray 3% Panchagavya ($0.34 \text{ mmol m}^{-2} \text{ s}^{-1}$), T_1 - Ambient ozone level ($0.34 \text{ mmol m}^{-2} \text{ s}^{-1}$) and T_5 - Ambient Ozone level + foliar spray 3% Neem oil ($0.29 \text{ mmol m}^{-2} \text{ s}^{-1}$) which were on par among themselves. Lowest stomatal conductance was observed elevated ozone exposure at 200ppb (T_3) ($0.14 \text{ mmol m}^{-2} \text{ s}^{-1}$) followed by T_{10} - Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya ($0.15 \text{ mmol m}^{-2} \text{ s}^{-1}$), T_2 - Elevated ozone exposure at 150ppb ($0.16 \text{ mmol m}^{-2} \text{ s}^{-1}$), T_{11} - Elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil ($0.16 \text{ mmol m}^{-2} \text{ s}^{-1}$), T_{12} - Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid ($0.18 \text{ mmol m}^{-2} \text{ s}^{-1}$) and T_7 - Elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya ($0.18 \text{ mmol m}^{-2} \text{ s}^{-1}$). After being exposed to different ozone levels, the ambient treatments with ozone protectants (Ascorbic acid, Neem oil and Panchagavya) were recorded to be increasing at the exposure intervals and followed by the ambient ozone without ozone protectants. The lowest values are found in the elevated levels of ozone (200ppb and 150ppb) followed by the treatment with elevated ozone level 200 ppb with ozone protectants and elevated ozone level 150 ppb with ozone protectants up to seven days.

3.1.3. Photosynthetic rate:

A significant reduction was observed in photosynthetic garlic rate due to elevated ozone levels from the first day after exposure (Table 2.). The highest photosynthetic rate was observed in ambient Ozone level + foliar spray 3% Panchagavya (T_4) ($4.68 \mu\text{mol CO}_2 \text{ s}^{-1} \text{ m}^{-2}$) followed by T_6 - Ambient Ozone level + foliar spray 0.1% Ascorbic acid ($4.62 \mu\text{mol CO}_2 \text{ s}^{-1} \text{ m}^{-2}$), which were on par. The lowest values were observed in elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil (T_{11}) ($2.23 \mu\text{mol CO}_2 \text{ s}^{-1} \text{ m}^{-2}$) and T_3 -

Elevated ozone exposure at 200 ppb ($2.29 \mu\text{mol CO}_2 \text{ s}^{-1}\text{m}^{-2}$), which were on par with T₁₂- Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid ($2.37 \mu\text{mol CO}_2 \text{ s}^{-1}\text{m}^{-2}$) and T₁₀- Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya ($2.44 \mu\text{mol CO}_2 \text{ s}^{-1}\text{m}^{-2}$). The lowest values on the final day were recorded in Elevated ozone exposure at 200 ppb (T₃) ($0.82 \mu\text{mol CO}_2 \text{ s}^{-1}\text{m}^{-2}$) followed by T₂- Elevated ozone exposure at 150 ppb (T₂) ($1.09 \mu\text{mol CO}_2 \text{ s}^{-1}\text{m}^{-2}$) compared to the elevated levels with ozone protectants. This showed that the ozone protectants significantly reduced the effects of ozone on garlic. Among the ozone protectants (panchagavya, neem oil and ascorbic acid) sprayed panchagavya (T₁₀- Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya and T₇- Elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya) and ascorbic acid (T₁₂- Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid and T₉- Elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid) decreased the effects of elevated ozone on garlic than neem oil.

3.2. Quality parameters

3.2.1. Pungency

There was a significant difference among the treatments for pungency content in garlic cloves due to elevated tropospheric ozone. Under the ambient condition, the highest value was recorded in Ambient ozone level + 0.1% Ascorbic acid ($74.29 \mu\text{mol g}^{-1}$) followed by Ambient ozone level + 3% Neem oil ($73.21 \mu\text{mol g}^{-1}$) treatment, Ambient ozone level + 3% panchagavya ($72.45 \mu\text{mol g}^{-1}$) treatment and Ambient ozone level ($59.73 \mu\text{mol g}^{-1}$). As the elevated levels of tropospheric ozone cause ill-effects to the crops, the treatment with Elevated ozone exposure at 200ppb ($38.93 \mu\text{mol g}^{-1}$) recorded the lowest value, followed by the treatment Elevated ozone exposure at 150ppb ($39.87 \mu\text{mol g}^{-1}$).

The ozone protectants help in nullifying the effects of ozone on the crop. In the garlic, the pungency in garlic bulbs after exposure with elevated levels of ozone with ozone protectants where the highest value recorded in the Elevated ozone exposure at 150ppb + 0.1% Ascorbic acid ($59.19 \mu\text{mol g}^{-1}$) followed by Elevated ozone exposure at 150ppb + 3% panchagavya ($58.97 \mu\text{mol g}^{-1}$), Elevated ozone exposure at 150ppb + 3% Neem oil ($54.71 \mu\text{mol g}^{-1}$). The lowest values are recorded in the treatment Elevated ozone exposure at 200ppb + 3% Neem oil ($44.65 \mu\text{mol g}^{-1}$) followed by the treatment Elevated ozone exposure at 200ppb + 3% Panchagavya ($47.85 \mu\text{mol g}^{-1}$) and Elevated ozone exposure at 200ppb + 0.1% Ascorbic acid ($50.57 \mu\text{mol g}^{-1}$).

3.2.2. Total Soluble Sugars

The total soluble sugars in the cloves of garlic showed significant differences among the treatments because of elevated tropospheric ozone in different concentrations were shown in Fig. 4. Among the treatments under normal conditions, the highest total soluble sugar content was recorded in Ambient Ozone level + foliar spray 3% Panchagavya (40.00°Brix) treatment followed by Ambient Ozone level + foliar spray 3% Neem oil (37.33°Brix) and Ambient Ozone level + foliar spray 0.1% Ascorbic acid

(37.33°Brix) treatment and the least value been recorded in Ambient ozone level (35°Brix) treatment. Since the elevated tropospheric level had a negative effect on the quality of garlic cloves, the lowest value was observed in Elevated ozone exposure at 200 ppb (28.00°Brix) treatment followed by Elevated ozone exposure at 150 ppb (29.67°Brix) treatment.

The application of ozone protectants had reduced the ill effect of elevated tropospheric ozone on the quality of garlic yield. Among the ozone protectant treatments, elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya (35.00°Brix) treatment had higher total soluble sugars followed by elevated ozone exposure at 150 ppb + foliar spray 3% Neem oil (34.00°Brix), elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid (31.67°Brix) treatment, elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya (31.33°Brix) treatment, Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid (31.33°Brix) treatment and elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil (30.00°Brix).

4. Discussion

The present study revealed that the physiology and quality of garlic were significantly affected by increased tropospheric ozone levels. The chlorophyll content of the garlic plant was significantly reduced by elevated ground-level O₃ of 150 and 200 ppb (69.67% and 77.29%) compared to the present ozone level. The significant chlorophyll reduction will undoubtedly affect the plant photosynthesis and reduce the garlic growth (plant height, above ground biomass- fresh and dry) and yield (number of cloves, diameter of bulb and weight of bulb) (Gayathri et al., 2019). The chlorophyll reduction might be due to the production of reactive oxidant species (ROS) in the apoplast by encountering apoplastic antioxidants and killing the cell. Our results, online with Kumari et al., 2013 reported a significant reduction in total chlorophyll content by 55.9% and a 50% reduction in total chlorophyll of soybean (Caregnato et al., 2013) under elevated ozone. The formation of superoxide radicals and hydrogen peroxide in leaf margin due to ozone exposure leads to trigger the injury signal by causing necrotic lesions and thus causes irreversible damage to the photosynthetic system that will lead to a reduction in photosynthetic rate.

Generally, progressive and irreversible reductions in photosynthetic activity occur upon acute O₃ exposure, and direct injury in leaf cells when leaves exposed to elevated ozone is also associated with the release of ubiquitous stress-induced volatiles such as volatile products of lipoxygenase (LOX) reactions (Li et al., 2017). Emissions of methyl salicylate (MeSA) was observed when the *Phaseolus vulgaris* plant exposed to acute dose of ozone whereas, the stomatal ozone uptake positively correlated with the total amount of emitted LOX products and methanol hence these results indicate that different stress volatiles scale differently with ozone dose and highlight the key role of stomatal conductance in controlling ozone uptake, leaf injury and volatile release (Li et al., 2017). The ozone-induced ROS formation in cantaloupe plants reduced by the production of superoxide dismutase will scavenge the superoxide (Zhang et al., 2020). The enzymatic and non-enzymatic leaf antioxidants cannot maintain the intercellular redox homeostasis due to higher ROS production in the cell, which leads to oxidative damage of membrane

lipids (Cargenato *et al.*, 2010). This disintegrated membrane can cause loss of chlorophyll a and b, which reduces the photosynthetic rate and biomass accumulation of Fepagro26 plants (Cargenato *et al.*, 2013)

The experimental results showed that the increasing ozone concentration reduced the stomatal conductance of the leaf (79.07% in 150 ppb and 86.05% in 200 ppb) from the first day after exposure. Though it is the immediate plant adaptive mechanism to escape from the ozone injury, the reduced stomatal conductance directly affects carbon dioxide exchange and transpiration rate, thereby reducing the growth and yield of garlic plants. In line with the findings of Noormets *et al.*, 2010, our results reported that when the plants were exposed to elevated ozone, they decreased the stomatal conductance, which reduced photosynthesis and internal CO₂ concentration. Under water stress condition lowered the *g_s* and thereby reduction in the entry of O₃ in to *B. nigra* plant caused less damage compared to well water condition at 550 ppb of ozone exposure. The cells were damaged and no recovery of *g_s* and lipoxygenase pathway observed, when the plant exposed to 550 ppb ozone level. At the same time the net assimilation rate also had decreased und water stress condition (Kask *et al.*, 2021).

Elevated O₃ caused visible injury and reductions in net photosynthesis, and these effects differed significantly among species. The deciduous species *Sorbari asorbifolia*, *Hibiscus syriacus* and *Fraxinus chinensis* were the most sensitive, while evergreen species ranked among the most tolerant. O₃ sensitivity was linked to both low leaf mass per area (LMA) and low leaf area-based antioxidant levels, but not to variation in leaf mass-based antioxidant levels or stomatal conductance. The well-known and easily measured leaf trait LMA thus represents a potentially useful metric for O₃ risk assessment and for selecting appropriate species for urban greening in O₃-polluted areas (Li *et al.*, 2016). In the palak (Lettuce) crop, the reduction in the capacity of the photosynthetic rate due to the leaf area reduction was observed under elevated ozone level compared to ambient ozone level (Kumari *et al.*, 2013). Similar results were observed in United States that 10% reduction in maize yield with current level of tropospheric ozone level. (Li *et al.*, 2019). The membrane integrity of the chloroplasts is affected by ozone, but afterwards, it affects mesophyll cells, which affect photosynthesis in this whole process. Hence, the effect on mesophyll cells brings both reductions in chlorophyll content, consequently leading to a reduction in photosynthesis rate (Tiwari *et al.*, 2018).

The quality parameter such as pungency (33.25%-150ppb and 34.82%-200ppb) and TSS (15.23%-150ppb and 20%-200ppb) had been reduced by increased ozone concentration. This reduction in quality could be due to a reduction in photosynthetic rate on one hand, where most of the available photosynthate diverted for antioxidant production metabolisms for sustaining the plant under elevated ozone concentration. Borberg *et al.*, 2015 and Frei *et al.*, 2015 revealed that the starch content and grain size were decreased in wheat and rice under elevated ozone. Recently similar results were observed in soybean by Broberg *et al.*, 2020 stating that the 200 kg/ha of seed protein was decreased due to current ozone levels. The altering plant composition and communities' structure due to elevated ozone, reduces the microbial biomass of soil, which in turn affects nutrient cycling process and reduction in Alpha diversity of the location (Agathokleous *et al.*, 2020). A recent meta-analysis investigated the effects of

ambient ozone on wheat by comparing yields in field-based open-top chambers (OTCs) ventilated with ambient air, with those in OTCs ventilated with filtered air that reduced the ozone concentration by on average 62%, effectively achieving pre-industrial concentrations (Pleijel *et al.*, 2018). Based on results from 33 experiments (from 9 countries, 3 continents using 17 cultivars plus one set of 4 cultivars), the average yield loss associated with reducing the mean ozone concentration from 35.6 to 13.7 ppb was 8.4%, with the starch and protein yield reduced by 10.9% and 6.2%, respectively. In the same meta-analysis, relative grain yield loss increased linearly as ozone concentration increased, with the highest losses being in the 20 - 30% range for some sites in India, China and the USA (Pleijel *et al.*, 2018).

The production of ascorbic acid by plants is a defence mechanism to alleviate ROS and allied stress. Exogenous application of ascorbic acid stimulates the growth and photosynthesis of wheat (Malik and Asraf, 2012). In many crops, the external application of ascorbate plays a vital role as an antioxidant. Some reports show that the external applications of ascorbate in plants reduced the foliar injury and improved photosynthetic rate against ozone impact ascorbate in apoplast can protect plants from ozone damage (Wang *et al.*, 2015; Dai *et al.*, 2020). Hence, applying ascorbic acid, neem oil, and panchagavya was performed to reduce the ozone impact on garlic crop in our study where nearly 10-20% amelioration took place against the effects of elevated levels of O₃. The plant species that produce an increased ascorbate level exhibit resistance to ozone exposure (Feng *et al.*, 2010). Yendrek *et al.*, 2015 revealed that garden pea showed the greater quantity of phenolic compounds, foliar glutathione and enhanced NADH dehydrogenase transcript abundance, which attributed the plants are highly resistant to ozone damage. Similarly, Venkatesh and Park, 2014 stated that ascorbate plays a significant role in cellular ROS scavenging activity and influences many stress responsive enzyme activities through synergetic action with other antioxidants such as glutathione and α -tocopherol to protect the cell from oxidative damage.

5. Conclusion

Hence, the present study revealed that the elevated tropospheric ozone showed deleterious effects on the physiology and quality of garlic, regulating the growth and yield. With the above observations, we conclude that the elevated tropospheric ozone with increased concentration showed detrimental effects on physiological responses (chlorophyll content, photosynthetic rate and stomatal conductance) and quality parameters such as pungency and total soluble sugars on garlic crop, nevertheless ozone protectants such as panchagavya, neem oil and ascorbic acid stimulated the plant defence mechanism, which generated the counteracting mechanism in protecting the plant tissues against O₃ toxicity. Among the ozone protectants, neem oil performed well followed by ascorbic acid and panchagavya. Though the tropospheric ozone is short-lived air pollutant, which have been identified as one of the potent greenhouse gas recently by Intergovernmental Panel on Climate Change (IPCC), future research may be focused to study the tropospheric ozone impact studies on different food crops in detail and identify the better ozone protectants and its mode of action for nullifying the negative impact of tropospheric ozone to sustain agriculture production and food security for an ever-increasing population with less land and limited water resources under changing climate.

Abbreviations

$^{\circ}\text{C}$	-	degree Celsius
ABL	-	Above Boundary Line
AOT	-	Accumulated Ozone Threshold
CH_4	-	Methane
CO_2	-	Carbon dioxide
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
Fig.	-	Figure
FRAP	-	Ferric Reducing Antioxidant Power
GBP	-	Geosphere Biosphere Program
GDP	-	Gross domestic product
Gs	-	stomatal conductance
HYSPLIT	-	Hybrid Single-Particle Lagrangian Integrated Trajectory
IPCC	-	Intergovernmental panel on Climate Change
ISRO	-	Indian Space Research Organisation
L	-	Litre
NADH	-	nicotinamide adenine dinucleotide dehydrogenase
ng	-	nano gram
O_3	-	Ozone
OTC	-	Open top Chamber
Ppb	-	parts per billion
ppm	-	parts per million
RCP	-	Representative concentration pathways
ROS	-	Reactive oxygen species

Declarations

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Ethical Approval	Not applicable
Consent to Participate	Not applicable
Consent to Publish	Not applicable
Authors Contributions	1. Gayathri JawaharJothi - corresponding author, conducted the field work, sample collection, analysis and final preparation of thesis and submitted 2. Boomiraj Kovilpillai developed and designed the methodology and performed entire correction of the work and finally shaped it. 3. Avudainayagam Subramanian was the chairman of the advisory committee 4. Jayabalakrishnan Raja Mani analyzed the air quality of the research location 5. Sudhir Kumar worked in correcting the manuscript All authors read and approved the final manuscript
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Availability of data and materials	1. The datasets analyzed during this current study are available from the corresponding author and it is a part of my post graduate thesis. 2. "The growth and yield of the garlic crop due to elevated levels of O₃" have added to the reference and its citation is "Gayathri J, Boomiraj K, Avudainayagam S, Maheswari M, Chandrasekhar CN, Karthikeyan S. Impact of tropospheric ozone on growth and yield of garlic in high altitude region of Western Ghats. Int J Chem Stud 2019;7(3):3099-3101". 3. The ambient O₃ data have retrieved from the project ISRO-GBP (ATCTM) which was an unpublished data. The project undergoing in our institution.

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Tables

Table 1. Influence of tropospheric ozone on stomatal conductance of garlic obtained by one-way ANOVA test for different treatments

Treatments	DBE	1 st DAE	3 rd DAE	5 th DAE	7 th DAE
T ₁ – Ambient ozone level	0.43 ^{dcba}	0.34 ^a	0.34 ^a	0.35 ^a	0.35 ^a
T ₂ – Elevated ozone exposure at 150 ppb	0.41 ^{dcba}	0.16 ^b	0.09 ^{cb}	0.13 ^b	0.09 ^b
T ₃ – Elevated ozone exposure at 200 ppb	0.31 ^{dc}	0.14 ^b	0.02 ^c	0.09 ^b	0.06 ^b
T ₄ – Ambient Ozone level + foliar spray 3% Panchagavya	0.33 ^{dcba}	0.34 ^a	0.35 ^a	0.37 ^a	0.41 ^a
T ₅ – Ambient Ozone level + foliar spray 3% Neem oil	0.27 ^d	0.29 ^a	0.36 ^a	0.38 ^a	0.42 ^a
T ₆ – Ambient Ozone level + foliar spray 0.1% Ascorbic acid	0.33 ^{dcba}	0.35 ^a	0.35 ^a	0.41 ^a	0.42 ^a
T ₇ – Elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya	0.35 ^{dcba}	0.18 ^b	0.14 ^b	0.16 ^b	0.09 ^b
T ₈ – Elevated ozone exposure at 150 ppb + foliar spray 3% Neem oil	0.41 ^{dcba}	0.19 ^b	0.09 ^{cb}	0.15 ^b	0.11 ^b
T ₉ – Elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid	0.41 ^{dcba}	0.20 ^b	0.14 ^b	0.17 ^b	0.13 ^b
T ₁₀ – Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya	0.54 ^a	0.15 ^b	0.09 ^{cb}	0.11 ^b	0.08 ^b
T ₁₁ – Elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil	0.51 ^{ba}	0.16 ^b	0.08 ^{cb}	0.14 ^b	0.07 ^b
T ₁₂ – Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid	0.46 ^{cba}	0.18 ^b	0.10 ^{cb}	0.14 ^b	0.09 ^b
P value (0.05)	0.041	0.000	0.000	0.000	0.000

(*DBE- Day before exposure, DAE- Day after exposure)

Table 2. Influence of tropospheric ozone on photosynthetic rate of garlic obtained by one-way ANOVA test for different treatments

Treatments	DBE	1 st DAE	3 rd DAE	5 th DAE	7 th DAE
T ₁ – Ambient ozone level	4.37 ^a	4.25 ^{ba}	3.65 ^a	3.31 ^{ba}	2.90 ^a
T ₂ – Elevated ozone exposure at 150 ppb	4.41 ^a	2.85 ^{cba}	2.39 ^{ba}	1.42 ^c	1.09 ^b
T ₃ – Elevated ozone exposure at 200 ppb	4.14 ^a	2.29 ^c	1.66 ^b	1.37 ^c	0.82 ^b
T ₄ – Ambient Ozone level + foliar spray 3% Panchagavya	4.68 ^a	4.68 ^a	3.89 ^a	3.96 ^a	3.02 ^a
T ₅ – Ambient Ozone level + foliar spray 3% Neem oil	4.58 ^a	4.25 ^{ba}	3.99 ^a	3.37 ^{ba}	2.75 ^a
T ₆ – Ambient Ozone level + foliar spray 0.1% Ascorbic acid	4.49 ^a	4.62 ^a	3.80 ^a	3.89 ^a	3.01 ^a
T ₇ – Elevated ozone exposure at 150 ppb + foliar spray 3% Panchagavya	4.75 ^a	2.93 ^{cba}	2.71 ^{ba}	2.43 ^{cb}	1.40 ^b
T ₈ – Elevated ozone exposure at 150 ppb + foliar spray 3% Neem oil	4.52 ^a	2.84 ^{cba}	2.77 ^{ba}	1.77 ^c	1.19 ^b
T ₉ – Elevated ozone exposure at 150 ppb + foliar spray 0.1% Ascorbic acid	4.69 ^a	2.87 ^{cba}	2.71 ^{ba}	2.38 ^{cb}	1.32 ^b
T ₁₀ – Elevated ozone exposure at 200 ppb + foliar spray 3% Panchagavya	4.44 ^a	2.44 ^{cb}	1.89 ^b	1.60 ^c	0.98 ^b
T ₁₁ – Elevated ozone exposure at 200 ppb + foliar spray 3% Neem oil	4.33 ^a	2.23 ^c	1.76 ^b	1.42 ^c	0.86 ^b
T ₁₂ – Elevated ozone exposure at 200 ppb + foliar spray 0.1% Ascorbic acid	4.37 ^a	2.37 ^{cb}	1.92 ^b	1.57 ^c	0.99 ^b
P value (0.05)	0.136	0.019	0.015	0.000	0.000

(*DBE- Day before exposure, DAE- Day after exposure)

Figures

Fig.1. Thermo Fischer U.S.A Model 49i – measuring ambient O₃ levels

Figure 1

See image above for figure legend.

Figure 2

O₃ Experimental design: (1) Potted garlic seedlings, (2) Garlic plants at the critical stage, i.e., bulb initiation stage, (3) Open-top chamber- within which the potted plants were exposed to elevated levels of O₃, (3a) Ozone Generator - fixed to the ground surface for stimulating elevated levels of ozone, (4) Ozone monitor- fixed inside the OTC for monitoring the O₃ elevated levels (150ppb and 200ppb), (5) Measurement of photosynthetic rate, stomatal conductance using Portable photosynthetic system and chlorophyll content by using chlorophyll content meter, (6) Potted garlic plants after exposure to elevated levels of O₃.

Figure 3

See image above for figure legend.

Fig. 4. Effect of tropospheric ozone on quality parameters of Garlic - Pungency ($\mu\text{mol g}^{-1}$) and Total Soluble Sugars ($^{\circ}\text{Brix}$) where Orange line indicates the pungency and Blue line indicates TSS ($^{\circ}\text{Brix}$).

Figure 4

See image above for figure legend.